Biopharmaceutical Development Program (BDP)

Bringing Projects from Laboratory Bench to Clinic: Development Challenges and Go/No-Go Decisions

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A subsidiary of Science Applications International Corporation
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Overview

Mission Statement

Our goal is the rapid translation of innovative scientific discoveries into therapeutic products that hold the real hope for preventing and curing cancer and other diseases.

The mission of the Biopharmaceutical Development Program (BDP) is to manage the technical operation of the NCI's Biological Resource Branch (BRB). The BDP assesses research and develops biopharmaceutical clinical products. The BDP interprets the current regulations and guidelines to produce "research grade" as well as "clinical grade" materials. The purpose is to explore "proof of principle" of novel therapeutic concepts, as well as early clinical trials for cancer and other diseases. The BDP performs technology transfer of developed products and processes to meet BRB initiatives.

BDP manufactures high-quality, laboratory-grade material to support preclinical development for selected innovative projects. The NCI selects and prioritizes candidate projects. BDP provides feasibility analyses and cost estimates that are used in the selection. We undertake a portfolio of projects based on the following criteria:

Program Approach

1. Concepts are selected for novelty and innovation over derivatives of existing approaches.
2. Many projects have been previously considered by Pharma and declined due to uncertain technology, regulatory hurdles, or small markets.
3. Clinical production is focused on meeting requirements for initial proof-of-concept trials, NOT final product requirements for commercial development.
4. Approximately 1/10 – 1/20 clinical projects may eventually be licensed.
5. Desired size of program is based on assumptions #1-4 (above), i.e., 10-20+ projects per year to clinic.
6. Intellectual Property to be retained by Project Originator(s).

Where Does the Program Get Projects?

1. Peer-reviewed extramural research
2. Special competitions:
   - NCI Rapid Access to Intervention Development (RAID) Program
   - NIDDK RAID for Type 1 Diabetes
   - Inter-Institute Program (IIP) for AIDS Projects
   - National Cooperative Drug Discovery Groups
3. Intramural NCI research
4. Other government programs (NIAID, NIDDK, USAMRIID)
5. Commercial collaborations with Government
6. Work for others

Program Capabilities

Mammalian Cell Culture

- 100-L and 1000-L Bioreactors
- Hollow Fiber Bioreactors
- CelliGen Plus® Bioreactors
- Wave Bioreactors
- Virus Production

Bacterial Fermentation

- New Brunswick 10-L and 20-L fermentation systems with Biocommand software control and on-line monitoring (Latchet) for process development studies
- 80-L NBS and 100-L B. Braun fermenters with (Clean in Place) CIP for cGMP fermentation operations with recombinant microorganisms
- 30-, 300-, 3000-gallon fermentation vessels for natural product fermentations
- Gaulin and Niro Homogenizers
- Continuous desludging centrifuge (Carr) processing tanks, crossflow filtration set up, evaporators

Protein Purification/Fill-Finish

- AKTA Explorers (Pharmacia) for purification development
- VirTis research freeze dryer
- Millipore and Pharmacia-automated purification skids for cGMP operation
- BPG columns of different sizes for cGMP operations
- Validated automated (3-ml and 5-ml) filling system (Monoblock)
- Automated labeling system

Selected Outsourcing

- Fill/Lyophilize: Formatech (Andover, MA)
- Adenoviral product for gene therapy: Q-1 Biotech (Glasgow, Scotland)
- Cell based vaccines: Bioreliance (Rockville, MD)

Analytical Development

- Cell based assays: cell based binding ELISAs, cell proliferation assays, FACS based assays
- Physico-chemical assays: HPLC with triple detector, cIEF, static and dynamic light scattering, circular dichroism
- BIAcore based assays
- Transfection assays

Biopharmaceutical Projects by Biological Resources Branch (1987-Present)

PROJECTS	AFFILIATION
Recombinant Protein Vaccines	
Mutant K-Ras Vaccines	NCI
F1-V Vaccine (Plague)	NIAID
rSEB Vaccine	USAMRIID
PA-83 (rPA) Vaccine (Anthrax)	USAMRIID/NIAID
hsp110 Melanoma Vaccine	Roswell Park Cancer Institute, NY
RTVP-1 Protein	Baylor College of Medicine
Viral Vaccines	
VEE Vaccine	USAMRIID/JVAP/Dynport
FGR Adenovirus Vaccine	Henry Ford Health System
AFP and GMCSF plasmids (2) and AFP Adenovirus Vaccines	Univ. of California, LA
TMVA-hvp53	City of Hope Medicial Center
Ad GMCSF-CAIX	David Geffen School of Medicine
Plasmid Vaccines	
PNGVL4pNGVL4a - Sig E7 (detox)/HSP70	Johns Hopkins Univ.
NGVL4a-CRT E7 Vaccine	Johns Hopkins Univ.
pING-TYRP2 Plasmid Vaccine	Memorial Sloan-Kettering
pWRG 1644 DNA Plasmid Vaccine	Univ. of Wisconsin
Vaccine - Cellular Products	
Pancreatic Tumor Cell Vaccines	Johns Hopkins Univ.
EBV Supernatant Vaccine	Johns Hopkins Univ.
Master Cell/Working Cell Bank (Allogeneic Vaccine)	Johns Hopkins Univ.
Phase I/II/III Id Vaccines (Lymphoma)	NCI
Vaccine Peptides	
PADRE-CMV Vaccine	City of Hope Medical Center
3 Vaccine Peptides (Breast Cancer)	Johns Hopkins Univ.
HPV 16 - E7/MET-9-THR	NCI
PCLUS 3-18MN Peptide	NCI
PR103 (p53-194 His) Vaccine	NCI
PR24 (ras-12 Arg)	NCI
PR26a/p53-282-T-P	NCI
PR-81 Peptide	NCI
PR86/p53-196Pro Peptide	NCI
PR92 (p53-144Arg)	NCI
PR95 (p53-159Pro)	NCI
V166A-VHL14	NCI
CAP-1 Peptide Vaccine	NCI
MART-1 Peptide Vaccine	NCI
HPV E7 Peptide Vaccine	NCI
Multiple Patient-Specific Peptide Vaccines	NCI
Khleif Peptide Vaccines	NCI
Peptide Vaccine	Thomas Jefferson Univ.
Bradykinin Antagonist Peptide	Univ. of Colorado Cancer Center
EF-1 Peptide	
PXFK Peptide	
Monoclonal Antibodies	
11B.11 Rat Anti-murine IL-4	NCI
1D12 Anti-hu IgM	NCI
2A11 Anti-bombesin	NCI
3 Antibodies, Bu12-Saporin, 4KB128-Saporin, OKT10-Saporin	The Flavell Leukemaia Research Unit
6D9 Anti-ID	NCI
7G7 Anti-IL-2 alpha R	NCI
9.3 Anti CD2 (transplantation)	Naval Medical Research Laboratory
95-5-49 Anti–T cell	NCI
95-6-22 Anti–T cell	NCI
Anti-CD45 MAB	Vanderbilt Univ.
Anti-CD54 Monoclonal Antibody	Univ. of Texas
B72.3 Anti-Tag 72	Univ. of Alabama, Birmingham
BC8	Vanderbilt Univ.
C-225 (Chimerization) Anti-EFGR	Univ. of California, San Diego
Ch 14.18 Anti-GD2	Univ. of California, San Diego
CH EB6 Anti-hu IgA	NCI
Ch R24 Anti-GD3	Memorial Sloan-Kettering
Murine R24 Anti-GD3	Memorial Sloan-Kettering
Murine E2.3 and A27.15 Anti-Transferrin Receptor	Univ. of Arizona Health Science
Chimeric 11-1F4 Anti-ID (Leukemia)	Univ. of Tennessee
D612 Anti–Colon Cancer	Univ. of Alabama, Birmingham
HD37	Univ. of Texas
HeFi-1	NCI, Harvard Medical School
HSV 863	NIAID
Hu 14.18 IL-2 Fusion Protein	Univ. of Wisconsin
Hu B3	NCI
Hu HB22.7	Univ. of California, Davis Cancer Center
Hu Mik Beta 1	NCI

PROJECTS	AFFILIATION
Monoclonal Antibodies (cont'd)	
huCC49-Delta-CH2 Anti-Tag 72	NCI
hoKT3γ1 (Ala, Ala)	NIDDK
MRK-16	
Mu B3	NCI
Murine 225 Production Anti–EFGR	Univ. of California, San Diego
Murine 42/6 IgA Anti-Transferrin Receptor	Univ. of Arizona Health Science
Murine 528 Anti-EFGR	Univ. of California, San Diego
Murine Anti-Tac Anti-IL-2R	NCI
Murine 11-1F4	Univ. of Tennessee
OVB3 Anti–Ovarian Antibody	NCI
RFB4	Univ. of Texas
VH4-34 IgM216	Stanford Univ. School of Medicine
PB3 (LEC-NH576)	Univ. of Southern California
Il-2 FcEIL-15 Fc	NIDDK
Natural Products	
Wortmannin	Arizona Cancer Center
Fumagillin	NCDDG
Geldanamycin	NCDDG
Staurosporin	NCDDG
Endotoxin	NCI
Immunotoxins	
MR1-1 DsFv-PE38	Duke Univ.
BL-22 (dsFv RFB4-PE38) (Lymphoma)	NCI
ERB-38	NCI
LMB-2 (Anti-TAC Fv PE-38)	NCI
HA-22 Immunotoxin (Lymphoma)	NCI
RN321-RFB4 Onconase	NCI
Recombinant Proteins	
Hu IL-7	Boston Univ. Hospital
Recombinant *Wolinella Asparaginase*	Indiana Univ.
Cyanovirin HIV Therapy	NCI
F-5 Cys Anti-Her2/neu scFv AB Targeted Liposome	Univ. of California, San Francisco
IL-4 Recertification	NCI
IL-6 Recertification	Johns Hopkins Univ.
IL-2 Recertification	NCI/Extramural
IL-15	NCI
Oligonucleotides	
KSR Antisense Oligonucleotide	Memorial Sloan-Kettering
c-myb Oligodeoxynucleotide	Univ. of Pennsylvania School of Medicine
Oncolytic Viruses	
Polio virus Chimera (Brain Tumors)	Duke Univ.
Ad 3/5 Delta-24	Helsinki Univ.
Delta-24 Adenovirus	M.D. Anderson
Oncolytic Virus Delta-24-RGD (Glioma and Ovarian Cancer)	M.D. Anderson
Ad5RGDCD/SSTR Oncolytic Virus Head & Neck Cancer	Univ. of Alabama, Birmingham
Herpes Vector Oncolytic Virus	Harvard-Massachusetts General Hospital
Ad5RGDTK/SSTRTK Oncolytic virus (Ovarian Cancer)	Univ. of Alabama, Birmingham
Tissue-specific CRAd: Ad Cox-2/RGD (Pancreatic Cancer)	Univ. of Alabama, Birmingham
Ad-CCL-21	Univ. of California, Los Angeles
MV-NIS (Multiple Myeloma)	Mayo Clinic, Rochester, MN
Ad-KD3	St. Louis Univ., St. Louis
Ad TSTA-Sr39tK (Prostate Cancer)	Univ. of California, Los Angeles
Cell Banks	
A549 Master Cell Bank	BDP Resource
Vaccinia Master/Virus Banks	BDP Resource
CHO Cell Bank	BDP Resource
MRC-5 Cells	BDP Resource
NS/O Cells	BDP Resource
VERO Cell Bank	BDP Resource
HEK293 Master Cell Bank	BDP Resource
K562 Cells	BDP Resource
2E8 Cells	SAIC-Frederick, Inc.
MVA Virus Bank	BDP Resource
T1165 Cells	BDP Resource
TF-1 Cells	BDP Resource
U87-MG Cells	BDP Resource
DH5 Alpha *E. Coli* bank	BDP Resource
***E. Coli* BL21AI**	BDP Resource
***E. Coli* BL21BRL**	BDP Resource

The projects that are bolded have been released for clinical use.
The italic projects have been released for toxicology.

Many Projects Require Substantial Attention at the Onset

Expression System Problems

- Errors in genetic sequence
- Extraneous genetic material
- Instability of genetic material
- Ampicillin selection pressure
- Lab-scale affinity purification only
- Protein solubility problems
- Low yield
- Poorly defined production system
- Inadequate purification schemes

Analytical Approaches

- Unvalidated or non-existent *in vitro* potency assay
- Lack of key reagents, e.g., antibodies to desired product
- Poor biochemical characterization

Regulatory and Safety

- Raw material qualification
- Inappropriate cell banks
- Difficult or unidentified toxicology systems
- Failed vendor qualification

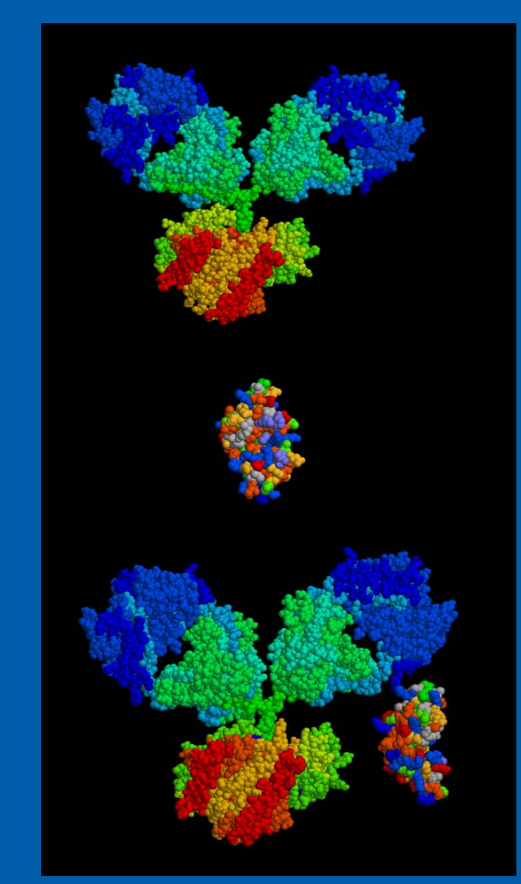
Other

- Intellectual property concerns
- Delays in Material Transfer Agreements (MTAs)
- Contracting delays
- Lack of synchronization between funding mechanisms
- Basic data cannot be reproduced

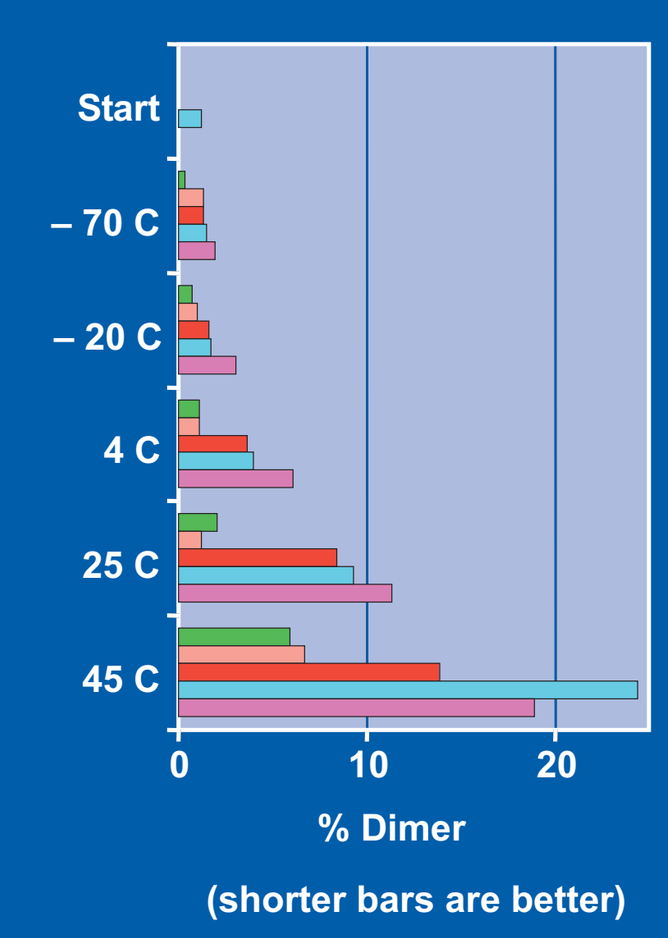
Aggregation: Formulation Solution

What is RFB4-Onconase?

- RFB4: A large, 140 kDa IgG, antibody that targets a carcinoma-related surface antigen (CD 22).
- Onconase: A small, 12 kDa, naturally occurring RNA-digesting protein having low systemic toxicity.
- RFB4-Onconase: RFB4 covalently linked to Onconase to produce an antibody-targeted, anti-cancer therapeutic.



Accelerated (4 day) Stability vs. Formulation



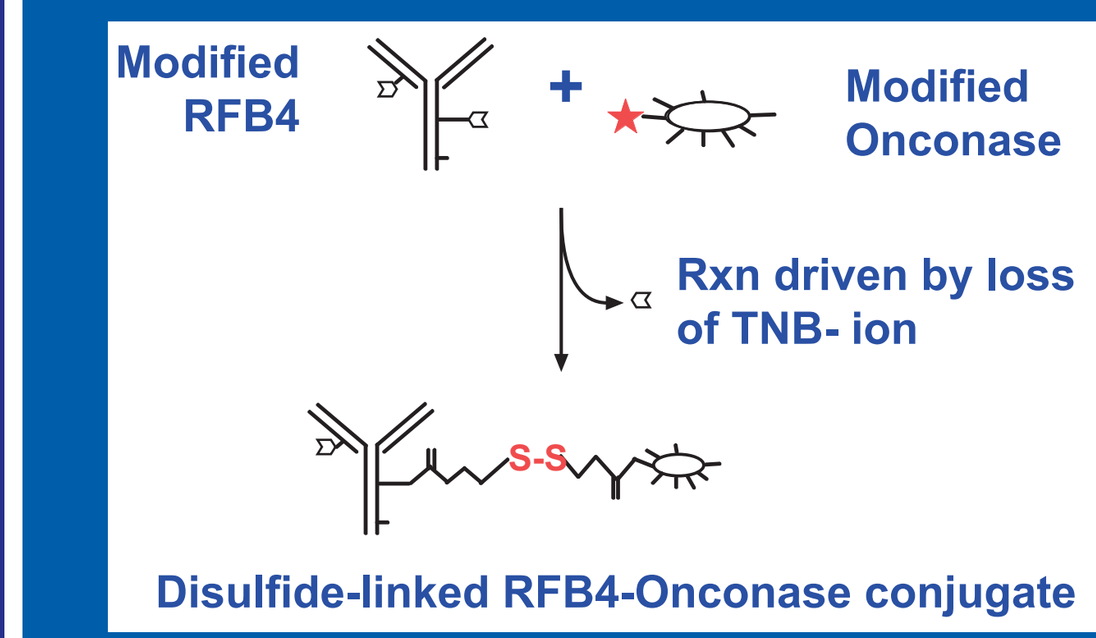
Study Performed with RFB4-RNaseA Surrogate

Free-thiol Scavengers:
- PBS, pH 7.4 + 0.2 mM N-Ethyl Maleimide
- PBS, pH 7.4 + 0.2 mM Iodoacetamide

pH Variation:
- PBS, pH 7.4
- PBS + Boric Acid to pH 8.5
- *PBS + Acetic Acid to pH 5.0*

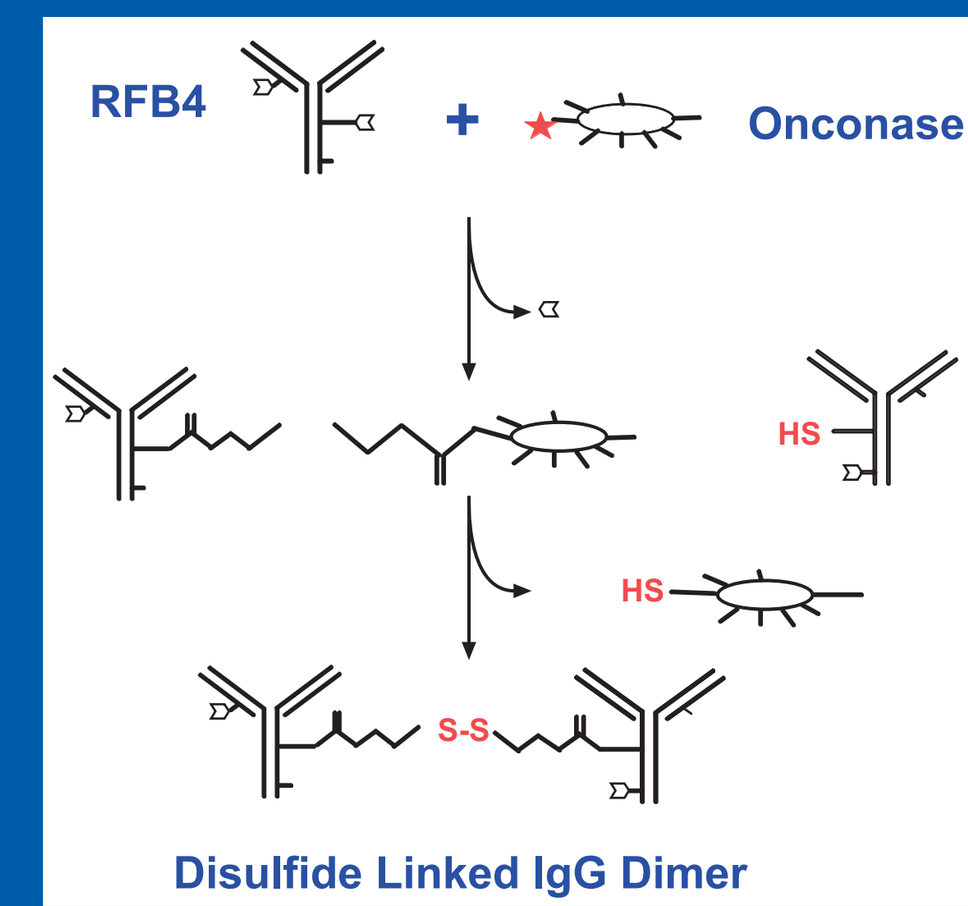
**** Best Condition ****

RFB4-Onconase Conjugation



Disulfide Dimer Type II

Disulfide Re-arrangement
- Faster at higher pH with free thiol catalysis.
- Suspected to be the primary mechanism acting in formulated conjugate.



RFB4-Onconase Instability

- RFB4-Onconase formulated to 1.5 mg/mL in PBS, pH 7.4.
- Stored at 2 to 8°C:
  - Within 24 hours, 8% dimer/multimer observed.
  - After 7 months, 38% dimer/multimer formed.
- Not acceptable for pre-clinical studies spanning several years.

Other projects that experienced similar issues:
- IgM
- RFB-Ricin

Stability Study Summary

Dimer formation was faster at higher pH/ temp.

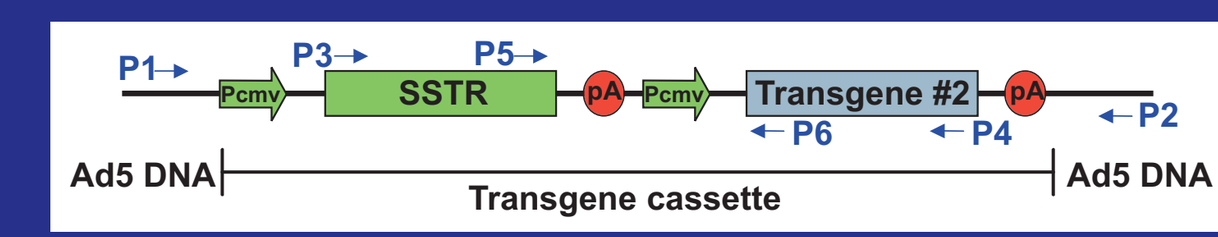
- RFB4-RNase solution stability was:
  - Unstable in PBS, pH 7.4
  - Modestly improved by IA
  - Significantly improved by NEM
  - Significantly Improved when held at pH (5.0).
  - Was maximally stable when stored at -70°a1C.
- This information was used to re-design the RFB4-Onconase formulation buffer.

Virus Instability

Viruses Found to be Unstable
- AdRGDTKSSTR
- AdRGDCDSSTR
- AdCDSSTR

Diagnostic PCR Analysis of Adenovirus Vectors Containing a Dual Expression Cassette

- Investigators designed and constructed a dual transgene cassette incorporated into an Adenovirus vector.
  - One gene (SSTR2-human somatostatin receptor subtype-2 gene) functions as an imaging tool.
  - The other gene (variable) delivers the therapeutic payload.
- The molecular design of the dual expression cassette resulted in the tandem repeat of both the promoter (CMV) and polyA tail (SV40) that regulate expression of the two genes:



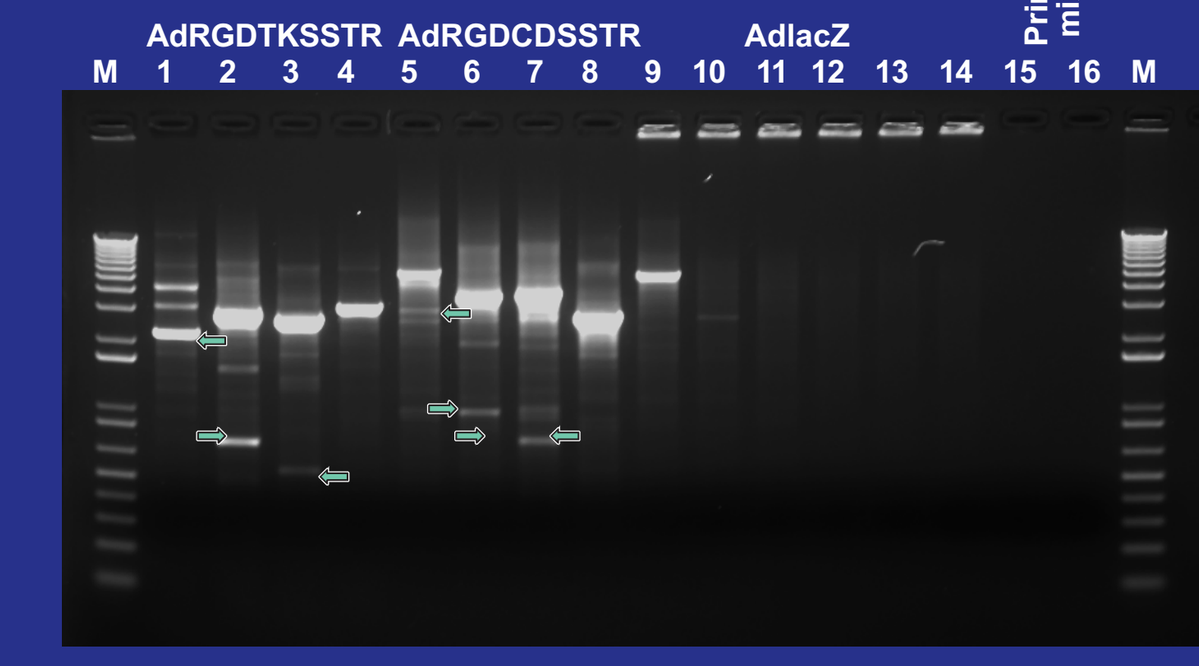
Observation During Molecular Characterization

- Attempts to amplify the complete cassette (using primers P1 & P2) generated both full-length product and deletion products, suggesting the deletion of one or other gene from the cassette. No such deletion products were observed using a control adenovirus, AdLacZ, carrying one inserted gene.
- Diagnostic PCR analysis using internal gene specific primers (P3-P6) supported this observation (see Table of Expected and Observed Amplification Product Sizes).

PCR Amplification Product Sizes

	Rxn	Primers	Predicted (FL) (approx. kb)	Deletion (approx.kb)	Observed (approx. kb)
Ad.RGD.TK.SSTR	1	Ad5 F278 (P1) + Ad 5 R3667 (P2)	4.1	2.2	4.1, (3.4), 2.2
	2	Ad5 F278 (P1) + HSVTK R150 (P6)	2.6	0.67	2.7, 0.67
	3	SSTR F1014 (P5) + Ad5 R3367 (P2)	2.6	0.63	2.6, 0.6
	4	SSTR F79 (P3) + HSVTK R1129 (P4)	3.0	N/A	3.0
Ad.CD.SSTR.RGD	5	Ad5 F278 (P1) + Ad 5 R3667 (P2)	4.1	2.2	5.5, 4.1, 3.0, 2.6
	6	Ad5 F278 (P1) + CD R562 (P4)	3.0	1.1	3.4, 0.95
	7	SSTR F1014 (P5) + Ad5 R3367 (P2)	2.6	0.6	3.5, 0.75
	8	SSTR F79 (P3) + CD R562 (P4)	2.4	N/A	3.5, 0.75
lacZ	9	Ad5 F278 (P1) + Ad 5 R3667 (P2)	4.9	N/A	5

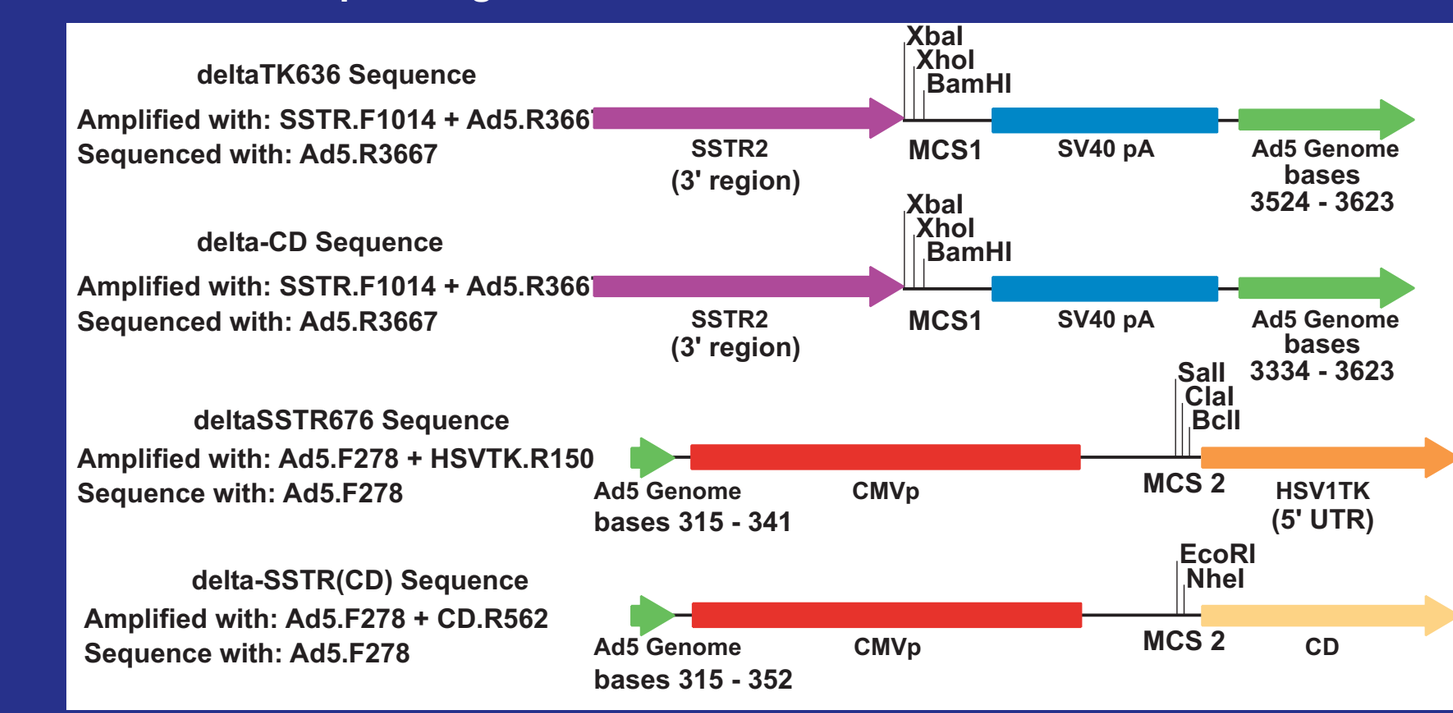
Gel Analysis of Amplification Products Observed



Arrows, ⇦, indicate potential deletion products

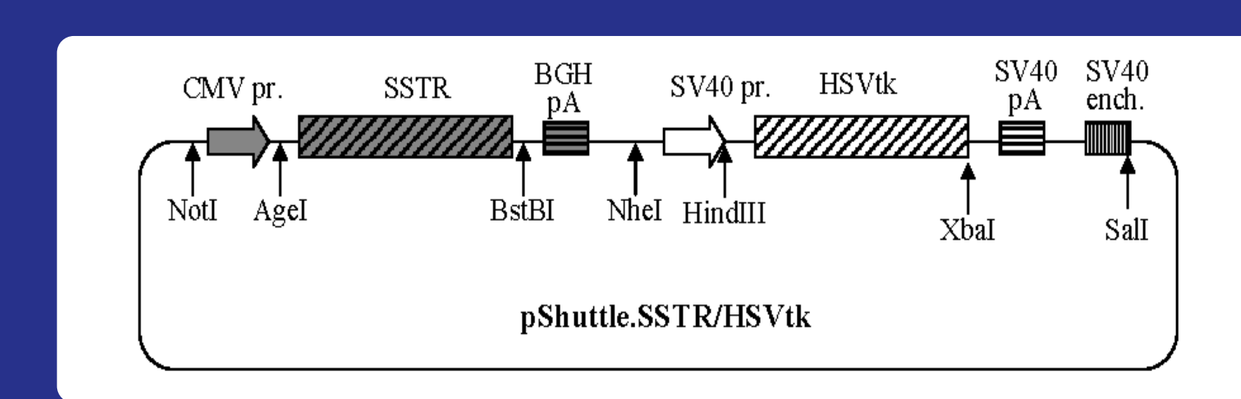
Further Molecular Characterization

Potential deletion products (from the highlighted reactions) were isolated and sequence analysis confirmed the existence of products with complete deletion of one or other gene mostly likely caused by homologous recombination between the repeated genetic elements.



Corrective Action (ongoing)

- These deletion events lead to the production of genetically unstable adenovirus vectors that are unsuited for clinical development.
- Investigators have redesigned their dual expression cassette to place each gene under the control of unique genetic transcriptional elements, eliminating DNA sequence duplication, which should minimize the potential for recombination leading to genetic instability:



Undesirable Mutations and Safety Issues

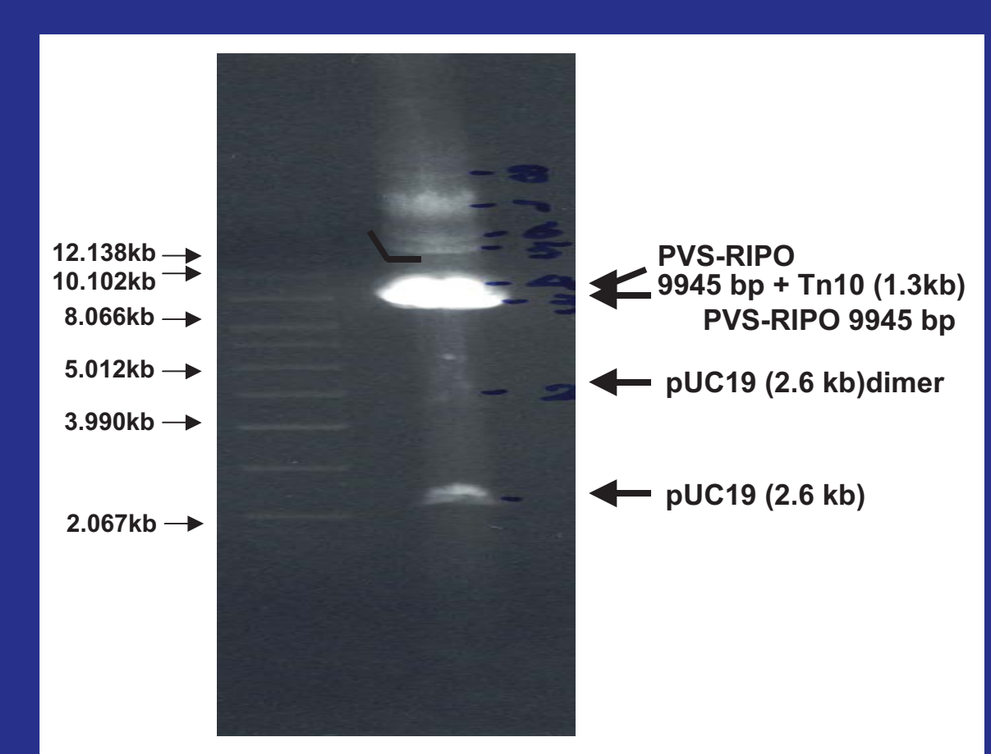
Product Development of PVS-RIPO: A Chimeric Poliovirus Vector for the Gene Therapy of Brain Tumors

Viral vectors have been extensively exploited for cancer gene therapy. Compared to DNA virus-based viral vectors, the development of RNA virus vectors poses unique challenges. The mutation rates in RNA viruses are about 1000-fold higher than that observed in DNA virus replication. Therefore, it is important to develop a process that ensures the safety and quality of RNA virus vectors.

A chimeric RNA virus, PVS-RIPO, was derived from the recombination of human rhinovirus and poliovirus (Sabin). This virus selectively replicates in, and kills, tumor cells of neuronal origin.

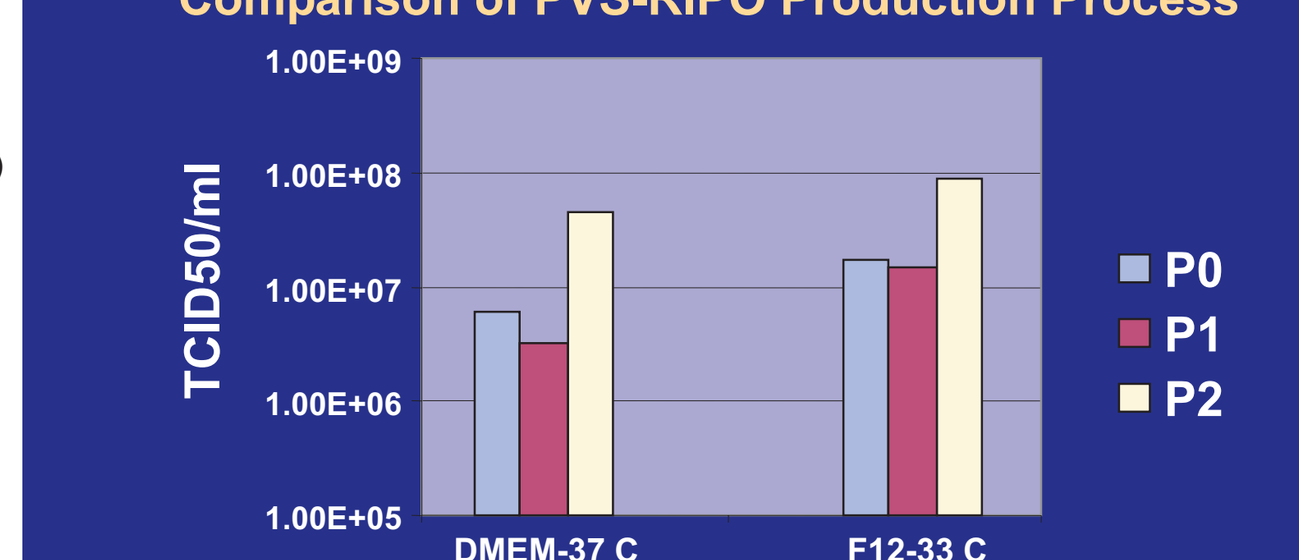
We present here the process development for the production of this novel viral vector for the treatment of brain tumors. Some of the problems and solutions are also discussed.

Plasmid PVS-RIPO DNA Starting Material



Electrophoresis analysis indicated that multiple products were present in the PVS-RIPO plasmid DNA starting materials, possibly due to the recombination events during the fermentation process.

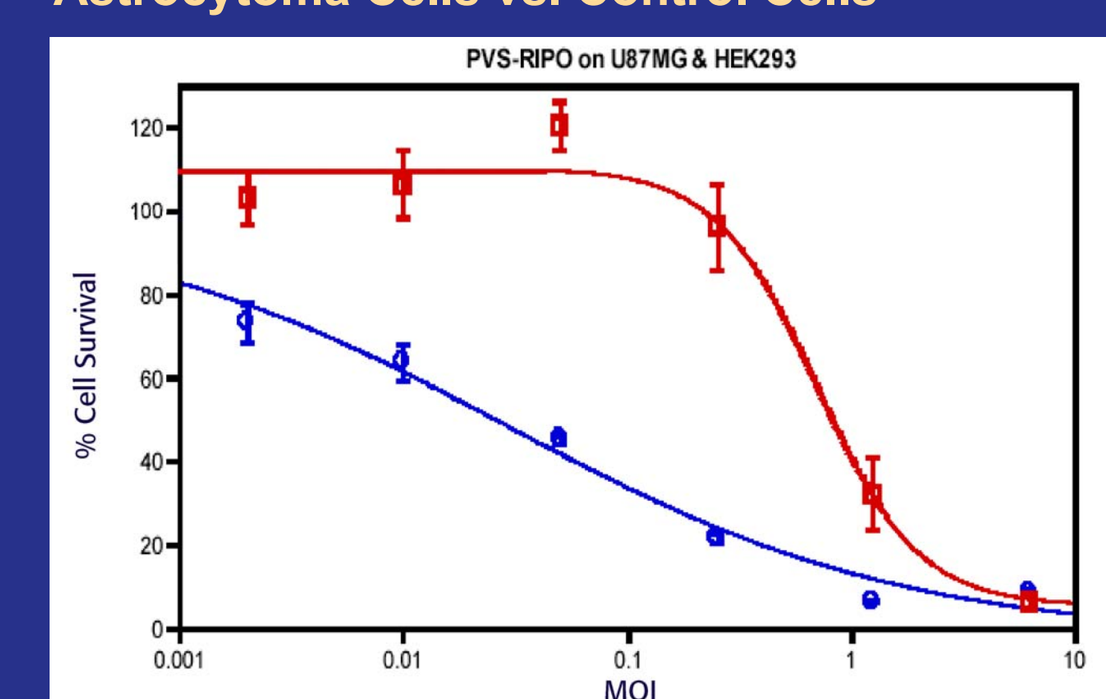
Comparison of PVS-RIPO Production Process



Conditions

Compared to the old production process (DMEM-37°C), the new process (F12-33°C) developed at the BDP, using serum-free medium, improved the overall virus yields significantly. We can now produce enough PVS-RIPO virus in just two rounds of virus infection (P1 and P2) in Vero cells. This limitation was required by the FDA due to the high mutation rates of this virus.

PVS-RIPO Virus Infection in Astrocytoma Cells vs. Control Cells



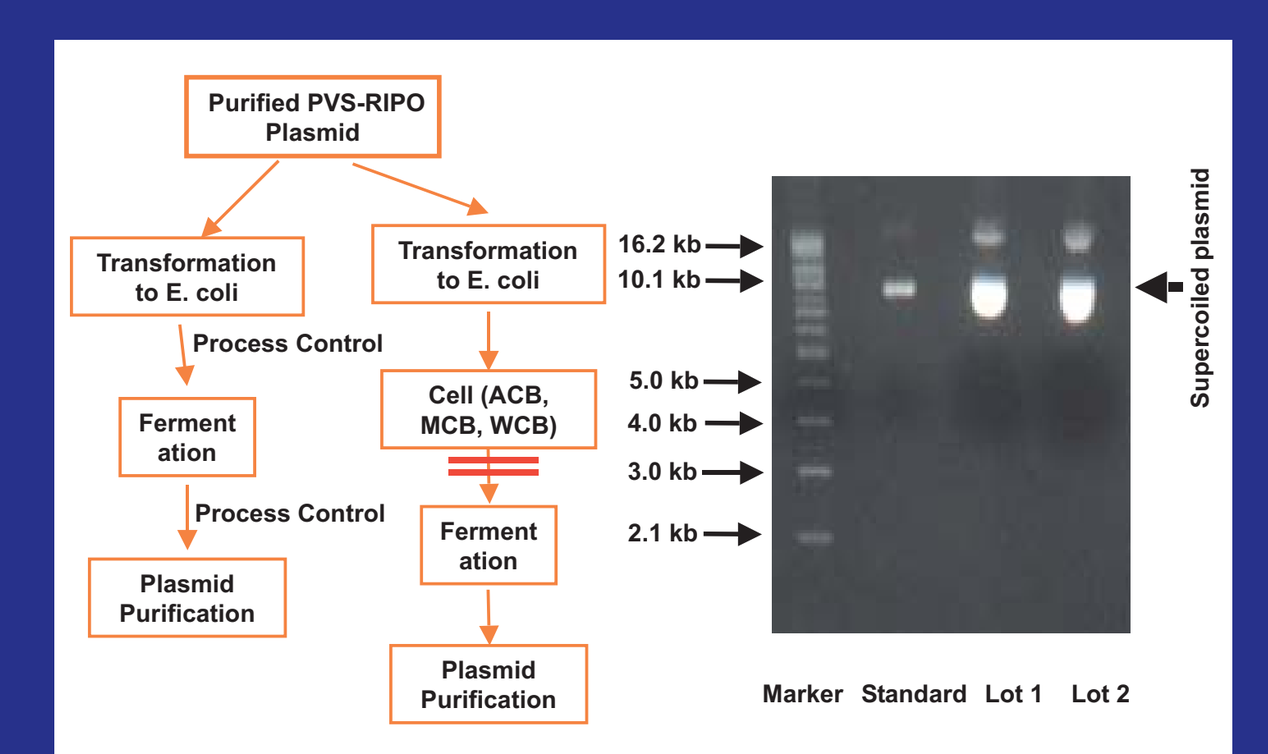
Astrocytoma cells (U87MG) and control cells (HEK293) were seeded in 96-well plates. After infection with PVS-RIPO virus at different concentrations, cell death was measured after addition of MTS dye reagent. U87MG cells were highly sensitive to PVS-RIPO infection, while HEK293 cells were relatively resistant to PVS-RIPO virus.

Production Process Flow Chart

Fermentation and Purification of DNA
⇩
Produce PVS-RIPO RNA through in vitro Transcription
⇩
Generation of Virus through Electroporation (P0)
⇩
Production of PVS-RIPO Viral Bank (MVB/WVB) (P1)
⇩
Production of PVS-RIPO Virus Harvest (P2)
⇩
Production of PVS-RIPO Bulk
⇩
Fill and Finishing

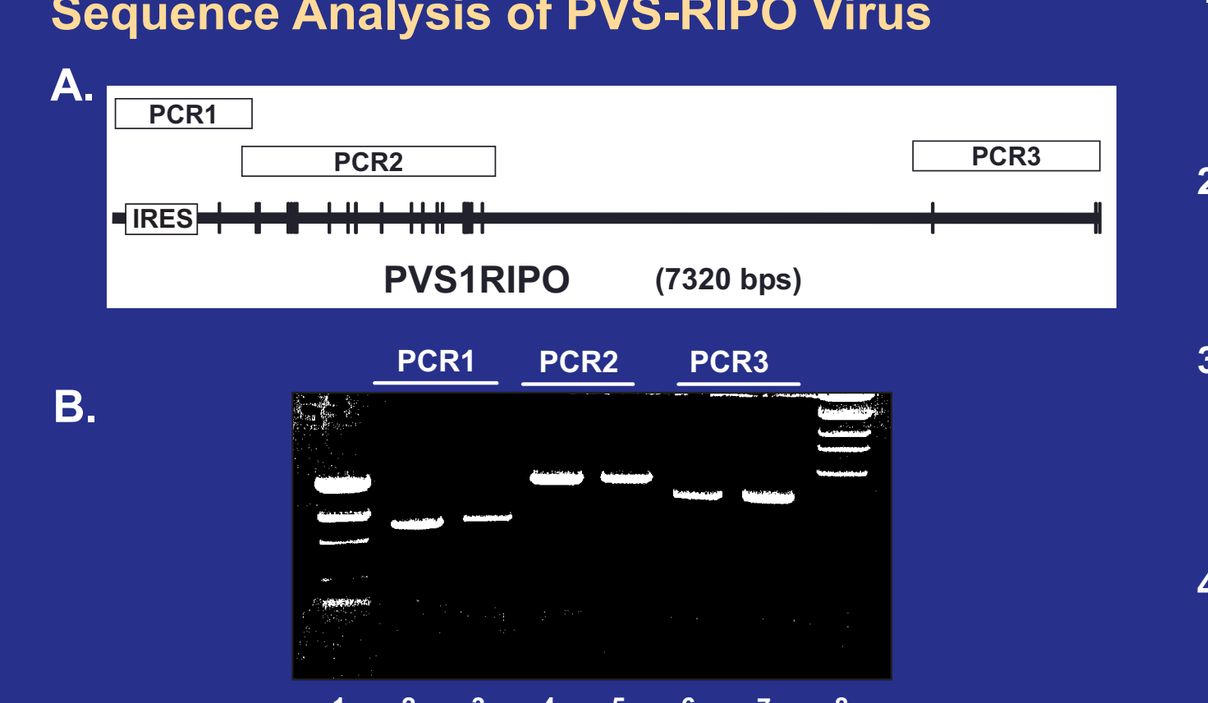
Other projects that experienced similar issues:
- D24 RGD RCA+ altered tropism
- rPA recombination of SPO gene from environmental anthrax

Plasmid PVS-RIPO DNA Production and Final Product



Due to its instability, PVS-RIPO plasmid DNA was purified from fermentation cultures immediately after transformation other than through the cell banking system. Correct plasmid DNA products were reliably obtained by this process.

Sequence Analysis of PVS-RIPO Virus



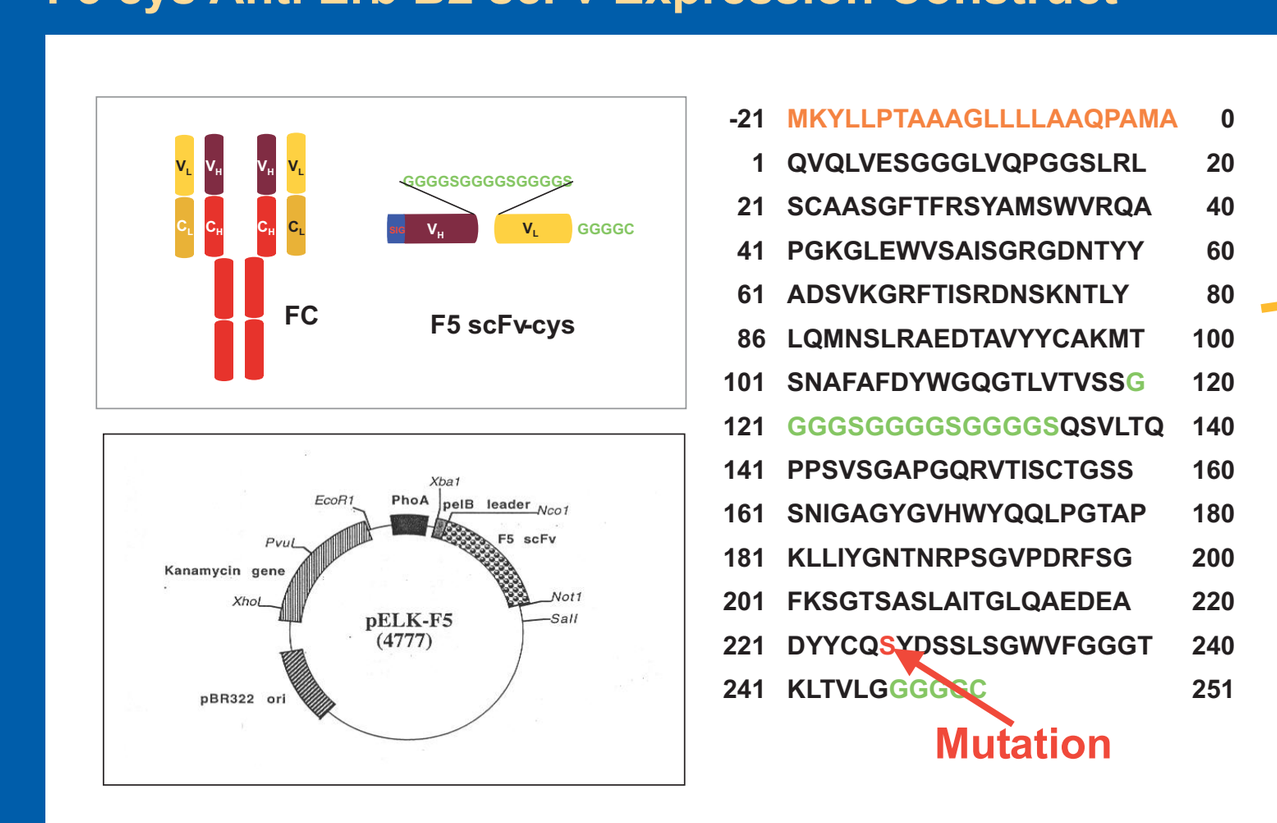
A. Neurovirulence determinants were spread over the PVS-RIPO virus genome, but were relatively concentrated in three regions (PCR1, PCR2, and PCR3).
B. Sequences in these regions of PVS-RIPO virus (P1) were analyzed by sequencing the DNA (Lanes 3, 5, and 7) after RT-PCR. The sequences were identical to that from PVS-RIPO virus reference materials (Lanes 2, 4, and 6). Lanes 1 and 8 were DNA ladders.

Conclusions

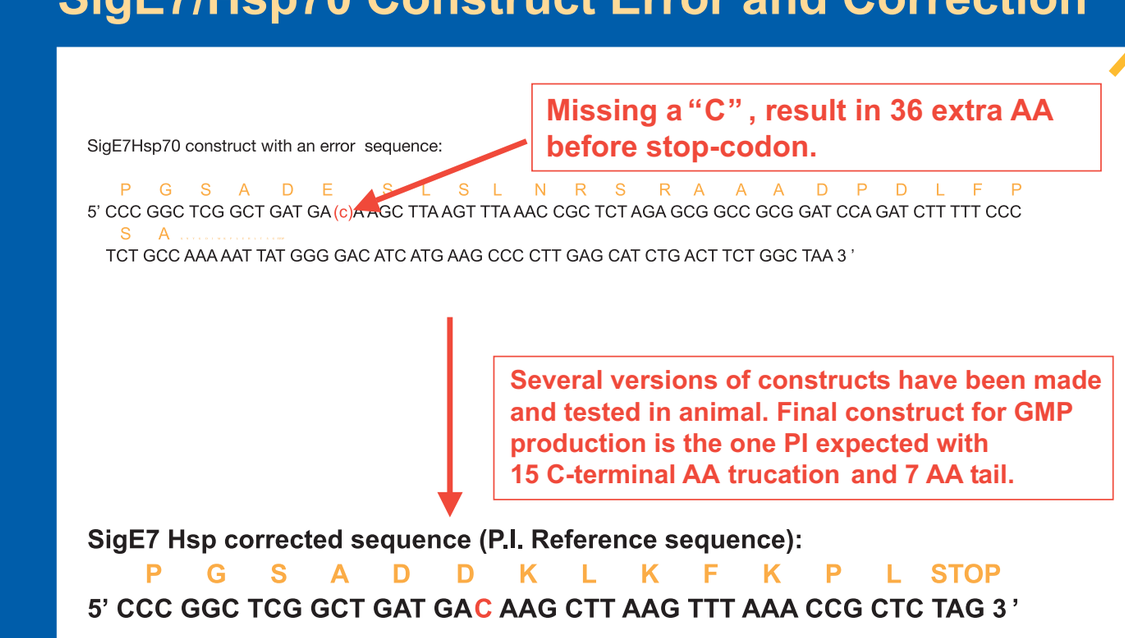
1. Plasmid harboring PVS-RIPO genome is not stable. However, a process was developed to amplify and purify the plasmid DNA.
2. A production process for PVS-RIPO viral vector consisting of steps of IVT, transduction, infection and purification was developed.
3. Viral deliverables can be achieved in two rounds of viral infection in Vero cells, thereby minimizing the safety concerns about this product due to its high mutation rates.
4. Molecular biology and biological assays indicated that the PVS-RIPO virus produced from this process had the expected sequence and selectively infected and killed brain tumor cells.

Sequence Errors: Plasmid Construction and DNA Sequence Problems

F5 cys Anti Erb B2 scFv Expression Construct



SigE7/Hsp70 Construct Error and Correction



Mutation in Asparaginase Expression Construct and Correction

There was a single-base mutation and this resulted in an amino acid residue change from Proline to Serine in the expressed protein.

Original clone	Final corrected clone
1224 "G"	1224 "C" – correct per Novagen
5429-30 "CT" ORF mutation	5429-30 "TC" ORF corrected per PI seq.
6210 "T"	6210 "C" – correct per Novagen

Both clones have:

458 "C", is T in Novagen ref seq

468 "deleted C" when compared to Novagen

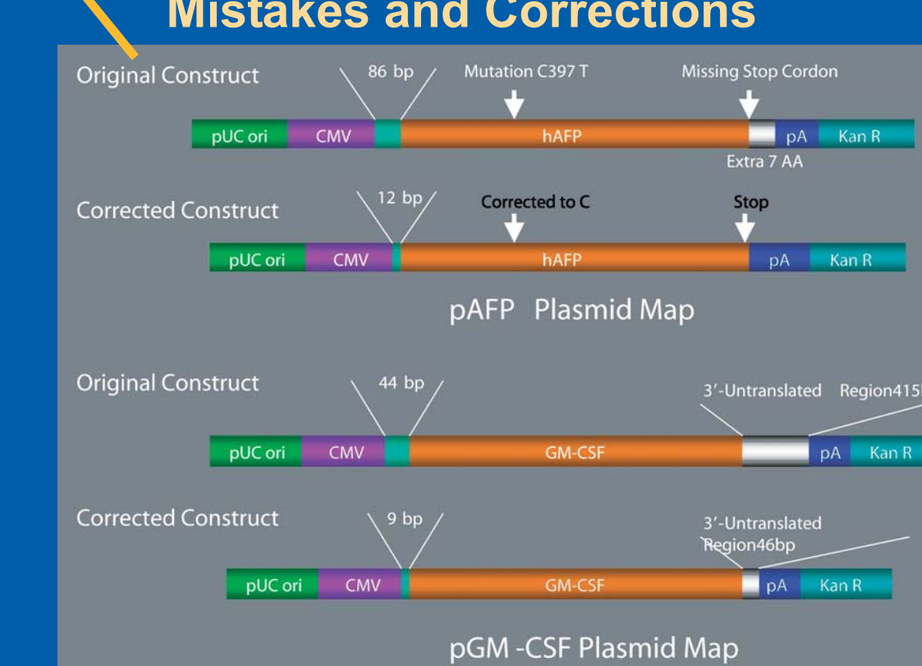
2319 "3 bp deletion" compared to Novagen

The mutations on the vector are not crucial.

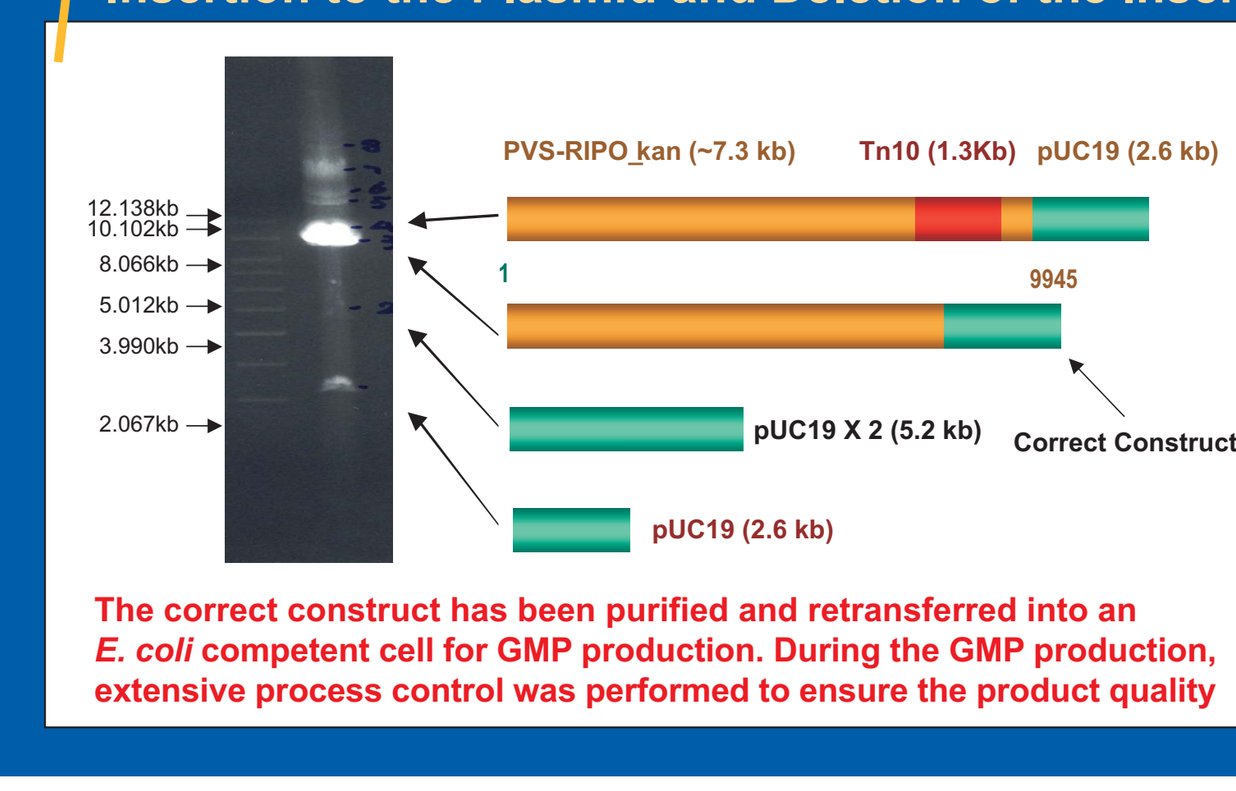
Summary of Plasmid Construction and DNA Sequence Problems in BDP Projects

Project	Product	Problem	Correction	Performed by
Staph. EntrotoxinB (#214)	SEB Vaccine	Construction mistake	Changed to Another Construct	PI
F5 cys scFv (#319)	F5 cys Single Chain Antibody	Point mutation Wrong sequence data	The mutated construct has been used for production	BDP
sigE7/Hsp70 (#390)	sigE7/Hsp70 PlasmidVaccine	Stop-codon missing	Three versions of 3egion have been constructed	BDP
Human Retinal S-antigen (#284)	S-antigen Vaccine	Three-point mutation	The mutations have been corrected	BDP
Asparaginase (#444)	Therapeutic Enzyme	Two-point mutations	Mutations have been corrected	BDP
Polio VirusPlasmid (#417)	Plasmidfor further viral production	E. coli DNA Insertion to the Plasmiddue to Instability of the Plasmid	Purified correcplasmid Quality control during GMP production	BDP
CRT/sigE7 (#471)	CRT/sigE7 PlasmidVaccine	Two-point mutations	Corrected mutations Animal tested with two constructs	BDP and PI
AFP/GM-CSF Plasmids (#447)	AFP and GM-CSF plasmids Vaccine	Point mutation and stop-codon missing 3' Un-translated region presented	Stop-codon has been added and un-translated region has been deleted	BDP

Plasmids pAFP and pGM-CSF Mistakes and Corrections



Plasmid PVS-PIRO Mutation by *E. coli* DNA Insertion to the Plasmid and Deletion of the Insert



The correct construct has been purified and retransferred into an *E. coli* competent cell for GMP production. During the GMP production, extensive process control was performed to ensure the product quality

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